510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083117

B. Purpose for Submission:
New Device

C. Measurand:
RNP70, Scl-70, and Jo-1 anti-nuclear antibodies

D. Type of Test:
Semi-quantitative fluoroenzyme immunoassay (ELISA)

E. Applicant:
Phadia US, Inc.

F. Proprietary and Established Names:
EliA™ RNP70 Immunoassay
EliA™ Scl-70 Immunoassay
EliA™ Jo-1 Immunoassay

G. Regulatory Information:
1. Regulation section:
   21 CFR§ 866.5100, Antinuclear Antibody Immunological Test System
2. Classification:
   Class II
3. Product code:
   LJM, Antinuclear antibody (enzyme-labeled), antigen, controls
   LKO, Anti-RNP antibody, antigen and control
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   See Indications for Use below.
2. Indication(s) for use:
   EliA™ RNP70 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to RNP70 in human serum and plasma (heparin, EDTA) as an aid in the clinical diagnosis of mixed connective tissue disease (MCTD) and systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA™ RNP70 uses the EliA™ IgG method on the instrument ImmunoCAP® 100 and ImmunoCAP® 250.

   EliA™ Scl-70 is intended for the in vitro semi-quantitative measurement of IgG

antibodies directed to Scl-70 in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of scleroderma (diffuse form) in conjunction with other laboratory and clinical findings. EliA™ Scl-70 uses the EliA™ IgG method on the instrument ImmunoCAP® 100 and ImmunoCAP® 250.

EliA™ Jo-1 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Jo-1 in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of polymyositis/dermatomyositis in conjunction with other laboratory and clinical findings. EliA™ Jo-1 uses the EliA™ IgG method on the instrument ImmunoCAP® 100 and ImmunoCAP® 250.

3. Special conditions for use statement(s):
   Prescription use only.
4. Special instrument requirements:
   ImmunoCAP 100 and ImmunoCAP 250 (k061165)

I. Device Description:

The EliA™ reagents are available as modular packages, each sold separately. The EliA™ wells are coated with human recombinant proteins (RNP70 (70 kD component), Scl-70, or Jo-1). The EliA™ wells are packed in carriers which are stored in sealed aluminum foil bags containing a desiccant.

The EliA™ Method-Specific reagents for ImmunoCap 100 or ImmunoCap 250 consists of: six levels of ready-to-use EliA™ IgG calibrators (0, 4, 10, 20, 100, 600 µg/L); IgG calibrator well (coated with mouse monoclonal antibodies); ready-to-use positive and negative controls; ready-to-use IgG curve control (20 µg/L); IgG conjugate (ß-Galactosidase anti-IgG mouse monoclonal antibodies) in PBS and; ready-to-use sample diluent (PBS with BSA). The EliA™ general reagents consist of: ready-to-use development solution (0.1% 4-Methylumbelliferyl-ß-D-galactoside); ready-to-use stop solution (4% Sodium Carbonate); ready-to-use 96-MicroWell™ plates; and ImmunoCap washing solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Varelisa U1RNP Antibodies
   Varelisa Scl-70 Antibodies
   Varelisa Jo-1 Antibodies
2. Predicate K number(s):
   k993589
   k944172
   k944173
3. Comparison with predicate:

EliA™ RNP70

Similarities		
Item	Device	Predicate
Intended Use	EliA™ RNP70 is intended for the in vitro semi-quantitative measurement of IgG antibodies	The Varelisa U1RNP Antibodies EIA kit is designed for the semi-

Similarities		
Item	Device	Predicate
	directed to RNP70 in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of mixed connective tissue disease (MCTD) and systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings.	quantitative and qualitative determination of U1RNP antibodies in serum or plasma to aid in the diagnosis of systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD).
Assay	ELISA	Same
Controls	EliA™ ANA Positive Control 100/250 EliA™ Negative Control 100/250	Positive and negative Control Sera included in the kit

Differences		
Item	Device	Predicate
Assay Type	Fluoroenzyme immunoassay	ELISA
Type of test	Semi-quantitative	Semi-quantitative and qualitative
Antigen	Human recombinant RNP 70 protein	Human recombinant U1RNP (RNP 70 kD, A, C)
Instrumentation	ImmunoCAP 100 and 250 are fully automated and integrated immunoassay analyzers	ELISA-Reader needed
Reaction Temperature	37°C controlled	Room temperature, 20-26°C
Detection Antibody	anti-human IgG ß-Galactosidase (mouse monoclonal antibodies)	anti-human IgG horse-radish peroxidase (goat)
Cut-off	Negative: < 7 U/mL Equivocal: 7-10 U/mL Positive: > 10 U/mL	Negative: < 5 U/mL Equivocal: 5-9 U/mL Positive: > 9 U/mL
Signal	Fluorescence	Optical density
Calibration	Total IgG Calibration	Analyte specific IgG Calibration
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	Calibration Curve in each assay
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit

EliA™ Scl-70

Similarities		
Item	Device	Predicate
Intended Use	EliA™ Scl-70 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Scl-70 in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of scleroderma (diffuse form) in conjunction with other laboratory and clinical findings.	The Varelisa Scl-70 Antibodies EIA kit is designed for the semiquantitative and qualitative determination of Scl-70 antibodies in serum or plasma to aid in the diagnosis of progressive systemic sclerosis.
Controls	EliA™ ANA Positive Control 100/250 EliA™ Negative Control 100/250	Positive and negative Control Sera included in the kit
Antigen	Human recombinant Scl-70 proteins	Same
Solid Assay	Polystyrene microwells	Same

Differences		
Item	Device	Predicate
Assay Type	Fluoroenzyme immunoassay	ELISA
Type of test	Semi-quantitative	Semi-quantitative and qualitative
Instrumentation	ImmunoCAP 100 and 250 are fully automated and integrated immunoassay analyzers	ELISA-Reader
Reaction Temperature	37°C controlled	Room temperature, 20-26°C
Detection Antibody	anti-human IgG ß-Galactosidase (mouse monoclonal antibodies)	anti-human IgG horseradish peroxidase (goat)
Cut-off	Negative: < 7 U/mL Equivocal: 7-10 U/mL Positive: > 10 U/mL	Negative: < 3 U/mL Equivocal: 3-8 U/mL Positive: > 8 U/mL
Signal	Fluorescence	Optical density
Calibration	Total IgG Calibration	Analyte specific IgG Calibration
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	Calibration Curve in each assay
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit

EliA™ Jo-1

Similarities		
Item	Device	Predicate
Intended Use	EliA™ Jo-1 is intended for the in vitro semi- quantitative measurement of IgG antibodies directed to Jo-1 in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of polymyositis/ dermatomyositis in conjunction with other laboratory and clinical findings.	The Varelisa Jo-1 Antibodies EIA kit is designed for the semi-quantitative and qualitative determination of Jo-1 antibodies in serum or plasma to aid in the diagnosis of polymyositis and dermatomyositis.
Controls	EliA™ ANA Positive Control 100/250 EliA™ Negative Control 100/250	Positive and negative Control Sera included in the kit
Antigen	Human recombinant Scl-70 proteins	Same
Solid Assay	Polystyrene microwells	Same

Differences		
Item	Device	Predicate
Assay Type	Fluoroenzyme immunoassay	ELISA
Type of test	Semi-quantitative	Semi-quantitative and qualitative
Instrumentation	ImmunoCAP 100 and 250 are fully automated and integrated immunoassay analyzers	ELISA-Reader needed
Reaction Temperature	37°C controlled	Room temperature, 20-26°C
Detection Antibody	anti-human IgG ß-Galactosidase (mouse monoclonal antibodies)	anti-human IgG horse-radish peroxidase (goat)
Cut-off	Negative: < 7 U/mL Equivocal: 7-10 U/mL Positive: > 10 U/mL	Negative: < 3 U/mL Equivocal: 3-8 U/mL Positive: > 8 U/mL
Signal	Fluorescence	Optical density
Calibration	Total IgG Calibration	Analyte specific IgG Calibration
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	Calibration Curve in each assay
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit

K. Standard/Guidance Document Referenced (if applicable):
None Referenced.

L. Test Principle:
The EliA™ wells are coated with a specific antigen. If antibodies that recognize that specific antigen are present in the patient's specimen they will bind. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies EliA™ IgG conjugate are added to form antibody-conjugate complexes. After incubation, non-bound conjugates are washed away and the bound complexes are incubated with a development solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators. The EliA™ IgG calibration is a total IgG calibration. It is based on a set of six WHO-standardized IgG calibrators derived from human serum. The calibrators are required to perform an initial calibration curve, which can be stored in the ImmunoCAP instrument and may be used up to 28 days. Each assay outside of a calibration run includes curve controls that have to fall within defined ranges to verify that the stored calibration curve is still valid.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. Precision/Reproducibility:
   Imprecision of the assays on the ImmunoCap 100 instrument was assessed by testing three serum samples with six replicates each, over 18 runs (3 instruments x 6 runs each, n = 108 replicates per sample). Imprecision of the assays on the ImmunoCap 250 instrument was assessed by testing three serum samples in triplicate over 21 runs (3 instruments x 7 runs each, n = 63 replicates per sample). A separate calibration curve was performed with each run.

Sample		ImmunoCAP 100			ImmunoCAP 250		
		Mean (EliA U/mL)	Intra-run (CV%)	Inter-run (CV%)	Mean (EliA U/mL)	Intra-run (CV%)	Inter-run (CV%)
EliA™ RNP70	1	10.6	6.8	1.4	5.9	4.9	5.7
	2	37.4	5.4	4.9	8.6	8.0	6.2
	3	44.7	4.1	4.7	56.3	4.9	6.5
EliA™ Scl-70	1	12.5	2.6	3.0	7.3	8.8	6.0
	2	45.3	3.1	3.2	34.6	4.5	4.9
	3	55.0	2.4	4.4	192.4	4.9	2.5
EliA™ Jo-1	1	12.3	2.7	3.8	8.2	5.4	5.9
	2	71.6	3.6	4.9	32.6	4.9	2.1
	3	77.0	2.3	5.4	105.5	5.3	4.7

   b. Linearity/assay reportable range:
   Percent Recovery:
   Three samples were further diluted from the method-specific 1:100 dilution according

to the following scheme: 1/1 (neat), 1/2, 1/4, 1/8, 1/16 and 1/32 using EliA™ Sample Diluent and the results are shown below. The samples were tested in two runs with three replicates and two replicates of the curve controls. The Observed/Expected (O/E)-values of the three samples for RNP70 were within the specifications. Not all O/E-values of the three samples for Scl-70 and Jo-1 were within the specifications (0.8 > O/E < 1.2) (out of specification values are highlighted in grey). However, due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range.

EliA™ RNP70		run 1			run 2		
Sample	Dilution	Obs mean (EliA U/mL)	Exp (EliA U/mL)	O/E	Obs mean (EliA U/mL)	Exp (EliA U/mL)	O/E
1	1/1	156.6			168.7		
	1/2	89.9	78.3	1.1	87.8	84.3	1
	1/4	43.8	39.2	1.1	44.3	42.2	1.1
	1/8	20.7	19.6	1.1	22.5	21.1	1.1
	1/16	10.3	9.8	1.1	10.3	10.5	1
	1/32	4.7	4.9	1	4.8	5.3	0.9
2	1/1	203.1			214.8		
	1/2	100.3	101.6	1	106.4	107.4	1
	1/4	53.6	50.8	1.1	53.6	53.7	1
	1/8	26.3	25.4	1	26.9	26.8	1
	1/16	12.6	12.7	1	13.3	13.4	1
	1/32	5.8	6.3	0.9	6.2	6.7	0.9
3	1/1	152.8			176.1		
	1/2	80.3	76.4	1.1	88.8	88	1
	1/4	41.2	38.2	1.1	43.8	44	1
	1/8	20	19.1	1	22	22	1
	1/16	9.8	9.5	1	10.4	11	1
	1/32	4.3	4.8	0.9	4.8	5.5	0.9

EliA™ Scl-70		run 1			run 2		
Sample	Dilution	Obs mean (EliA U/mL)	Exp (EliA U/mL)	O/E	Obs mean (EliA U/mL)	Exp (EliA U/mL)	O/E
1	1/1	181.3					
	1/2	101.7	90.7	1.1	101.9	90.1	1.1
	1/4	49.7	45.3	1.1	49.6	45.0	1.1
	1/8	25.2	22.7	1.1	25.0	22.5	1.1
	1/16	12.8	11.3	1.1	11.6	11.3	1.0
	1/32	6.6	5.7	1.2	5.9	5.6	1.0
2	1/1	141.2			144.1		
	1/2	67.8	70.6	1.0	68.5	72.1	1.0
	1/4	31.8	35.3	0.9	32.5	36.0	0.9
	1/8	17.1	17.7	1.0	15.5	18.0	0.9
	1/16	8.6	8.8	1.0	7.7	9.0	0.9
	1/32	4.4	4.4	1.0	3.9	4.5	0.9
3	1/1	118.1			118.0		
	1/2	62.1	59.0	1.1	52.4	59.0	0.9

	1/4	26.5	29.5	0.9	25.1	29.5	0.9
	1/8	12.5	14.8	0.8	11.4	14.7	0.8
	1/16	6.3	7.4	0.9	5.5	7.4	0.7
	1/32	3.0	3.7	0.8	2.8	3.7	0.8

EliA™ Jo-1		run 1			run 2		
Sample	Dilution	Obs mean (EliA U/mL)	Exp (EliA U/mL)	O/E	Obs mean (EliA U/mL)	Exp (EliA U/mL)	O/E
1	1/1	82.3			77.6		
	1/2	54.8	41.1	1.3	52.1	38.8	1.3
	1/4	29.1	20.6	1.4	28.7	19.4	1.5
	1/8	13.5	10.3	1.3	14	9.7	1.4
	1/16	6.1	5.2	1.2	6.3	4.9	1.3
	1/32	2.7	2.6	1	2.8	2.4	1.2
2	1/1	92.6			87.1		
	1/2	64	46.3	1.4	60.7	43.6	1.4
	1/4	34.9	23.2	1.5	33.5	21.8	1.5
	1/8	16.8	11.6	1.4	17.2	10.9	1.6
	1/16	7.2	5.8	1.2	7.3	5.5	1.3
	1/32	3.2	2.9	1.1	3.1	2.7	1.1
3	1/1	95.6			88.4		
	1/2	66.3	47.8	1.4	63.2	44.2	1.4
	1/4	39.3	23.9	1.6	37.1	22.1	1.7
	1/8	18.9	12	1.6	19.1	11.1	1.7
	1/16	9.7	6	1.6	9.7	5.5	1.8
	1/32	4.5	3	1.5	4.6	2.8	1.7

The measuring range (detection limit, upper limit) for EliA™ RNP70 and Jo-1 is from 0.3 to ≥ 240 EliA U/mL, and for Scl-70 is 0.4 - ≥ 320 EliA U/mL. The measuring range for each analyte is claimed from the detection limit to upper limit and is defined by the formula: (*highest calibrator point*) x (*conversion factor from reference batches*) x (*factor to assure a minimum of measuring range*). The EliA system measuring range is fixed and given in µg/L from 0-600µg/L. However, the use of batch specific factors will convert the different upper limits of the measuring ranges to? U/mL. The conversion factors from reference batches are: 0.5 for EliA RNP70 and EliA Jo-1, and 0.66 for EliA Scl-70.

The lot specific factor is determined on reference batches in a way that the cut-off, (which was determined in µg/L) is assigned the value of 10 EliA U/mL for all EliA ANA tests. In routine production new batches are compared with reference batches and if a difference is seen, then the batch-specific conversion factor is adjusted so that the cut-off remains at 10 EliA U/mL.

The factor to assure a minimum of measuring range is set at 0.8 for EliA RNP70, EliA Scl-70 and EliA Jo-1. Due to the batch-specific conversion factors, it is not possible to determine a fixed upper limit for the measuring range and it is noted in the labeling that the upper limit of the reported results can vary due to a lot-specific

conversion from µg/L to EliA U/mL. Results above the stated upper limit are reported as "above". They also state that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
IgG calibrators are traceable (via an unbroken chain of calibrations) to the International Reference Preparation (IRP) 67/86 of Human Serum Immunoglobulins A, G and M from WHO. New batches of IgG Calibrators are compared to a secondary standard (standardized with the IRP) or the IRP directly and adjusted accordingly to meet the correct concentration. The instrument measures specific IgG concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA ANA Wells, the results are automatically converted to EliA U/mL.

d. *Detection limit:*
In order to determine the lower limit of the measuring range (detection limit), the ability of the new device to differentiate between serial dilutions of Calibrator 4.0 and background (sample diluent on EliA™ antigen wells) was evaluated. Calibrator 4.0 (4 ug/L IgG) was serially diluted with EliA sample diluent, in halves to 0.5 ug/L (1:8), and run on EliA IgG calibrator wells and compared to the Calibrator results. The discrimination ability of the assay is represented by "D" and acceptance criterion was set as: D should be >2.0 for calibrator 4 diluted 1:4 (i.e. 1 µg IgG/L) on the different EliA™ antigen wells for both the detection limit and hook effect.

EliA™ RNP70

Results on Calibrator Wells			
Dilution	Mean Response Units [RU]	SD [RU]	D
1:2	260	6.1	42.5
1:4	162	2.9	56.6
1:8	116	2.6	45.2
Results on RNP70 Wells			
Sample Diluent	0	0	

EliA™ Scl-70

Results on Calibrator Wells			
Sample ID	Mean Response Units [RU]	SD [RU]	D
1:2	254	8.5	29.9
1:4	156	31	50.1
1:8	115	2.4	47.5
Results on Scl-70 Wells			
Sample Diluent	0	0	

EliA™ Jo-1

Results on Calibrator Wells			
Dilution	Mean Response Units [RU]	SD [RU]	D
1:2	230	2.2	98.8
1:4	131	3.2	40.5
1:8	91	2.5	35.2

Results on Jo-1 Wells			
Sample Diluent	0	0.6	

Both the RNP70 and Jo-1 were able to discriminate samples containing 0.5 µg/L IgG (Cal 4.0 1:8) from the background. The corresponding detection limit was 0.25 EliA U/mL when using 0.5 as correction factor to convert µg/L to EliA U/mL.

The EliA™ Scl-70 was able to discriminate samples containing 0.5 µg/L IgG (Cal 4.0 1:8) from the background. The corresponding detection limit was 0.33 EliA U/mL when using 0.66 as correction factor to convert µg/L to EliA U/mL.

Hook effect:
Hook effect was analyzed by using dilutions from high positive serum samples with an estimated concentration high above calibrator 600 (containing 600 µg/L of IgG). The sample dilutions were measured in 4 replicates.

	RNP70		Scl-70	
	Cal 600	High pos. sample (1:100)	Cal 600	High pos. sample (1:10)
Mean [RU] est.	20254	24368	19900	22087
SD [RU]	374.8	138.3	426.7	204.2
CV [%]	1.9	0.6	2.1	0.9
D		10.3		4.6
Times highest calibrator point		7		25

For Jo-1, no hook effect data were provided due to the inability to locate sera showing signals higher than the highest calibrator point (*i.e.,* 600 µg/L.

e. Analytical specificity:

Interference:
For each assay, a low positive or equivocal sample and a high positive sample were diluted with sample diluent and spiked with different amounts of interfering substances or their respective blank solutions, and analyzed in triplicates. A calibration curve was run in duplicate. Acceptance criterion of the ratios of spiked sample vs. spiked blank of 0.8 to 1.2 was established. A calibration curve was run in duplicate. The runs were repeated twice. One batch of EliA™ antigen wells and one batch of system reagents were used throughout the studies. The following final concentrations of additives in the samples were reached:

Bilirubin C – 1,930 mg/dL
Bilirubin F – 2,100 mg/dL
Chyle – 95,000 Units/dL
Hemoglobin – 47,000 mg/dL
Rheumatoid Factor IgM – 4,800 IU/mL

There was no demonstrated interference with any of the assays at these concentrations except for Bilirubin C and Hemoglobin in EliA™ RNP70. With Bilirubin C, ratios of

0.7 and 0.4 were observed with both samples for both runs, respectively. One sample (low), for run 1, gave a ratio of 0.7 for Hemoglobin but was within specification for the second run. The second sample was within specification for both runs. Generally, the use of sera that are lipemic, hemolyzed or have microbial contamination is not recommended as stated in the package inserts (Section: Specimen collection, Handling and Preparation).

Cross-reactivity:
A panel of the International Reference Panels for autoantibodies from the Centers of Disease Control (CDC) and Association of Medical Laboratory Immunologists (AMLI) were tested in duplicate using one batch of the EliA assays. The CDC panel included 10 samples identified as positive for dsDNA, ssDNA, Histone, (weak) Sm, SS-B/La, (weak) SS-A/Ro, Centromere, U1RNP, Scl-70, and Jo-1. The AMLI panel included 8 positive samples and 2 negatives. The positive samples contained both major antibodies as well as other minor antibody levels and contained the same antibodies as the CDC with the exception of Histone. Patient diagnosis for the CDC samples wasn't available, however the AMLI samples included: CREST, scleroderma, MCTD, Sjögren's syndrome, polymyositis, SLE, and healthy.

With one exception the new devices detect the targets as expected. Pertaining to EliA RNP70, CDC5 was found positive/equivocal with the test, but is reported to be high positive for Sm and histone only. In nature, however, Sm is associated with RNP and a parallel detection of the two antigens is not unexpected?. The results demonstrated a good specificity and sensitivity for the single tests. No cross-reactivity to other autoantibodies could be detected.

f. Assay cut-off:
The sponsor tested 400 apparently healthy Blood Donor samples from Caucasian individuals equally distributed by sex and age to evaluate expected values in the normal population and to confirm the previously defined cut-offs. The sponsor's acceptance criteria for validating the cut-offs was that the 95th percentile should lie below the lower limit of the equivocal range and the 99th percentile should lie below the upper limit of the equivocal range.

Test	Equivocal Range (ELiA U/mL)	Mean (ELiA U/mL)	95thPercentile (EliA U/mL)	99th Percentile (EliA U/mL)
EliA RNP70	7 – 10	0.9	1.8	2.3
EliA Scl-70	7 – 10	0.1	0.3	0.5
EliA Jo-1	7 – 10	0.1	0.3	0.4

2. Comparison studies:

a. Method comparison with predicate device:
Samples known to be positive or negative for respective ANA antibodies were analyzed together with a calibrator curve run in duplicate. Calibrators and Controls of the predicate device were also analyzed in duplicates. Test results were evaluated according to the description in the corresponding Directions for Use. For the

calculation of Positive Percent Agreement, Negative Percent Agreement and Overall Agreement, equivocal results were excluded.

RNP70

One hundred ninety-two serum samples were collected from the serum bank at Phadia GmbH. The study included samples from patients who had been clinically defined as suffering from Mixed Connective Tissue Disease (MCTD) (n=50) and Systemic Lupus Erythematosus (SLE) (n = 46). Disease controls consisted of: Rheumatoid Arthritis (RA) (n = 17), infections (n = 45), monoclonal gammopathy (n = 19), Tumor (n = 14), and unknown (n = 1).

		Varelisa U1RNP			
		Positive (>9 U/mL)	Equivocal (5-9 U/mL)	Negative (<5 U/mL)	Total
EliA RNP70	Positive (>10 U/mL)	36	0	2	38
	Equivocal (7-10 U/mL)	1	0	0	1
	Negative (<7 U/mL)	19	15	120	154
	Total	56	15	122	193

Technical Agreement

		Varelisa U1RNP		
		Positive (>9 U/mL)	Negative (<5 U/mL)	Total
EliA RNP70	Positive (>10 U/mL)	36	2	38
	Negative (<7 U/mL)	19	120	139
	Total	55	122	177

Positive Percent Agreement	65% (36/55)	51.4 – 77.8 ($CI_{95\%}$)
Negative Percent Agreement	98.4 (120/122)	94.2 – 99.8
Overall Agreement	88.1 (156/177)	82.4 – 92.5

EliA Scl-70

One hundred fifty serum samples were collected from the serum bank at Phadia GmbH. The study included samples from 50 patients who had been clinically defined as suffering from progressive systemic scleroderma (PSS)and Disease controls: 25 Rheumatoid Arthritis (RA), 50 infections, 9 Vasculitis, 10 Wegener's Granulomatosis, 4 Panarteritis nodosa, and 2 Churg-Strauss-Syndrome.

		Varelisa Scl-70			
		Positive (>8 U/mL)	Equivocal (3-8 U/mL)	Negative (<8 U/mL)	Total
EliA Scl-70	Positive (>10 U/mL)	25	0	0	25
	Equivocal (7-10 U/mL)	0	1	0	1
	Negative (<7 U/mL)	2	1	121	124
	Total	27	2	121	150

Technical Agreement

		Varelisa Scl-70		
		Positive (>8 U/mL)	Negative (<3 U/mL)	Total
EliA Scl-70	Positive (>10 U/mL)	25	0	25
	Negative (<7 U/mL)	2	121	123
	Total	27	121	148

Positive Percent Agreement	92.6% (25/27)	75.7 – 99.1 ($CI_{95\%}$)
Negative Percent Agreement	100 (121/121)	97.0 – 100.0
Overall Agreement	98.6 (146/148)	95.2 – 99.8

EliA Jo-1

One hundred forty-five serum samples were collected from the serum bank at Phadia GmbH. The study included patients who had been clinically defined as suffering from polymyositis/dermatomycsitis (PM /DM) were included. 45 Myositis (including Polymyositis and Dermatomyositis), disease controls: 25 Rheumatoid Arthritis (RA), 50 infections 9 Vasculitis, 10 Wegener's Granulomatosis, 4 Panarteritis nodosa, and 2 Churg-Strauss-Syndrome.

		Varelisa Jo-1			
		Positive (>8 U/mL)	Equivocal (3-8 U/mL)	Negative (<8 U/mL)	Total
EliA Jo-1	Positive (>10 U/mL)	18	4	3	25
	Equivocal (7-10 U/mL)	0	1	0	1
	Negative (<7 U/mL)	0	0	119	119
	Total	18	5	122	145

Technical Agreement

		Varelisa Jo-1		
		Positive (>8 U/mL)	Negative (<3 U/mL)	Total
EliA Jo-1	Positive (>10 U/mL)	18	3	21
	Negative (<7 U/mL)	0	119	119
	Total	18	122	140

Positive Percent Agreement	100% (18/18)	81.5 – 100.0 ($CI_{95\%}$)
Negative Percent Agreement	97.5 (119/121)	93.0 – 99.5
Overall Agreement	97.9 (137/140)	93.9 – 99.6

b. Matrix comparison:

The suitability of different sample matrices for each assay was determined by collecting serum, EDTA, lithium heparin and citrate plasma samples from fifty different donors. Sample values spanned the range of the assays. Negative samples did not switch to positive in any serum/plasma combination in any assay. Linear regression of the equivocal and positive samples for each serum/plasma combination in each assay demonstrated that the matrices were equivalent.

	Slope	95% CI	Intercept	95% CI
RNP70				
Serum vs. EDTA plasma	1.083	1.034 – 1.144	-0.712	-1.388 – 0.000
Serum vs. Li Heparin plasma	0.921	0.853 – 0.977	0.258	-1.247 – 3.45
Scl-70				
Serum vs. EDTA plasma	1.052	1.035 – 1.072	-0.226	-1.635 – 0.197
Serum vs. Li Heparin plasma	1.010	0.977 – 1.049	-0.656	-1.623 – 0.795
Serum vs. citrate plasma	0.986	0.961 – 1.050	-0.188	-1.820 – 1.092
Jo-1				
Serum vs. EDTA plasma	0.933	0.904 – 0.966	-0.035	-1.471 – 1.294
Serum vs. Li Heparin plasma	0.909	0.881 – 0.963	0.708	1.421 – 1.840
Serum vs. citrate plasma	0.857	0.824 – 0.899	1.220	-0.642 – 2.351

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

EliA RNP70

One hundred forty-five samples consisting of patients with MCTD and 95 disease controls were assessed to demonstrate sensitivity and specificity of the EliA RNP70.

		MCTD		
		Positive	Negative	Total
EliA RNP70	Pos. (>10 U/mL)	30	2	32
	Neg. (<7 U/mL)	20	93	113
	Total	50	95	145

Sensitivity: 60% (30/50) (45.2 – 73.6% $CI_{95\%}$)
Specificity: 97.9 (93/95) (92.6 – 99.7% $CI_{95\%}$)

EliA Scl-70

One hundred fifty samples consisting of 50 patients with PSS and 100 disease controls were assessed to demonstrate sensitivity and specificity of the EliA Scl-70. .

		PSS		
		Positive	Negative	Total
EliA Scl-70	Pos. (>10 U/mL)	23	2	25
	Neg. (<7 U/mL)	27	98	125
	Total	50	100	150

Sensitivity: 46% (23/50) (31.8 – 60.7% $CI_{95\%}$)
Specificity: 98% (98/100) (93 – 99.8% $CI_{95\%}$)

EliA Jo-1

One hundred forty-five samples consisting of 45 patients with Myositis and 100 disease controls were assessed to demonstrate the sensitivity and specificity of EliA.

		Myositis		
		Positive	Negative	Total
EliA Jo-1	Pos. (>10 U/mL)	25	0	25
	Neg. (<7 U/mL)	20	100	120
	Total	45	100	145

Sensitivity: 55.6% (25/45) (40.0 – 70.4 $CI_{95\%}$)
Specificity: 100% (100/100) (96.4 – 100% $CI_{95\%}$)

4. Clinical cut-off:
   Not applicable.
5. Expected values/Reference range:
   See assay cut-off.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.